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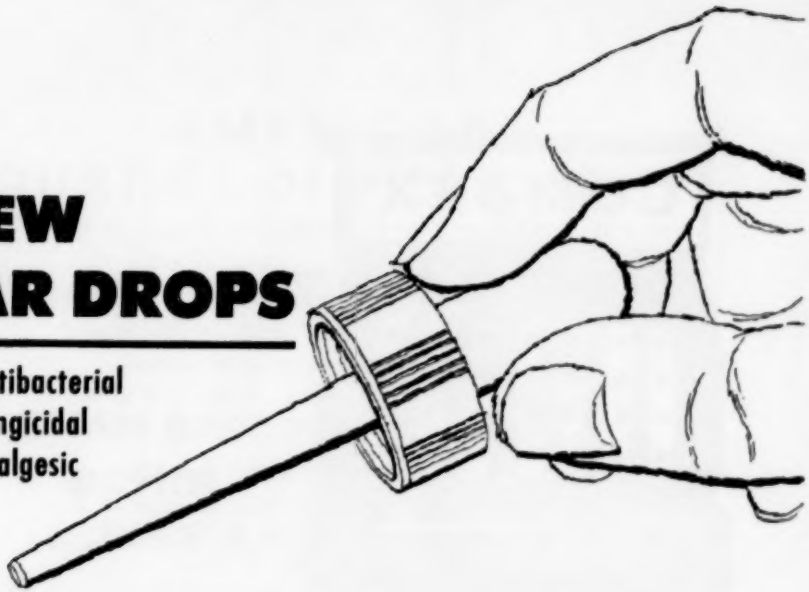

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EDITORIAL

THE FIFTIETH ANNIVERSARY OF THE FOOD AND DRUGS ACT

IT IS hard today for one to appreciate the chaotic conditions which prevailed in foods and drugs before the passage of the Pure Food and Drugs Act of 1906. The literature of the years prior to that date —if read today—would seem like a "chamber of horrors" in comparison with modern day standards and practices.

In commemorating the fiftieth anniversary of this landmark in food and drug legislation, it is quite fitting that we pay tribute to the late Dr. Harvey W. Wiley. It was he whose pioneering work culminated in this first national act to protect consumers against the then excesses and irresponsibilities of manufacturers and suppliers of foods and drugs. Dr. Wiley was not only a great scientist but he knew how to translate his findings into action. He took his message to the public and essentially stumped the country, arousing people from their complacency and acquainting them with the constant threats to their health and welfare which heretofore had been almost ignored. His dynamic leadership and crusading spirit slowly but surely won him many supporters. Finally, even some of those responsible for adulterated, misbranded, and harmful foods and drugs "got religion" and gave him their support.

Only those who have tried to arouse public opinion and obtain positive corrective action can appreciate the great effort and personal sacrifices which such a militant crusade involves and, while we this year honor Dr. Wiley, we must remember that he was roundly condemned and hated by many of those whose practices he sought to correct. It is ever thus with many great men. When history and future generations finally give them acclaim, we are inclined to remember the great good which was achieved but overlook the frustrations, heartaches, and downright punishment which were endured in bringing about their great accomplishment.

Dr. Wiley had other scientists who supported him and helped build up the mass of evidence needed to bring about the passage of the Act and, in paying tribute to him, their contributions should not be overlooked. The late Dr. Charles H. LaWall was such a supporter and we well remember in our early days hearing him recount in the lecture hall many of his personal experiences in food and drug work. That men like Dr. LaWall were towers of strength in giving assistance to Dr. Wiley can well be appreciated. They, too, knew quite well both their laboratory techniques and the fine art of translating their observations into public understanding. This, indeed, is a gift not possessed by all scientists, but one which makes the holder of inestimable value to both his field of science and to the public.

We have come a long way since the original act of 1906 and our present Food, Drug and Cosmetic Act with its Amendments is a model for the civilized world. Possibly, no other country has a better law and gives its people better protection.

The situation today between government and industry is a far cry as compared with that which existed fifty years ago. The food and drug industries have grown to become partners with government in protecting the consumer, and no responsible company, today, will knowingly do something which is potentially harmful to the public. The attitude of food and drug officials has likewise changed. Their educational functions now far outweigh their police action, although this at times must still be taken. The complexities of modern day practices make the task of the Food and Drug Administration a stupendous one and it is unfortunate that this government agency is hard-pressed at times to convince Congress of its proper needs. There seems, however, to be a very definite trend in recent years to appreciate more fully the significance of the service rendered the American people by this organization. Our legislators are beginning to appreciate that, as a matter of simple logic, we should be ready and willing to spend as much to protect our own health as we spend in giving economic aid to some far distant country where, at times, this aid brings us doubtful returns.

In summary, we pay tribute to the late Dr. Wiley, endorse fully the celebration this year of the Golden Anniversary of his famous Act, and commend all those who today carry on his great traditions.

L. F. Tice

SOME ASPECTS OF CHEMICAL "STERILIZATION" OF INSTRUMENTS

By Emil G. Klarmann [1]

Introduction

IS sterilization of surgical instruments and related objects possible of achievement by *chemical* means? In raising this question it is first necessary to agree on the definition of the term "sterilization." As used in its proper i.e., all-inclusive meaning, this term denotes elimination from the sterilized object of all microbial life, and especially of resistant bacterial spores.

As is well known, of all the microorganisms, certain bacterial spores offer the greatest resistance to destruction by physical or chemical means. It is also known that, with regard to this resistance, marked differences exist not only between the different species of spore-bearing bacteria, but also between the spores of a given species. While this is not the place to consider the subject of spore-bearing pathogens in its many ramifications, certain relevant findings merit consideration with specific reference to the hospital background of this study.

While, as a general rule, one does not encounter any extensive contamination with bacterial spores of surgical instruments following their practical use, the occurrence of such contaminants is by no means rare. Thus Roberts, Johnson, and Bruckner (1) found *Clostridium welchii* on 8.5 percent of scalpels used on a presumably clean skin, while Brewer (2) reported the presence of anaerobes on 12 per cent of blades used in surgery; however, in neither case was an actual spore count carried out. Spaulding (3) made an attempt to obtain an idea as to the number of spores present under these conditions by examining blades dipped in specimens of surgical pus. He found that their number ranged from 0 to 15, with an average of 2 per blade. However, higher numbers of spores may be expected to occur on instruments used on "dirty" infected wounds, and still higher ones on those coming in contact with fecal matter in the course of bowel surgery (4).

1. Lehn & Fink, Inc., New York, N. Y.

All this would indicate that the need for the destruction of spores on surgical instruments will vary somewhat with the case upon which the instruments have been used. However, where such a need has been established, the procedure employed must be effective under the pertinent practical conditions of use, i.e., it must be capable of sterilizing the instrument in the true sense of this term, as defined above. The evidence for the ability to effect such sterilization must be supported by representative tests, employing adequate or even exaggerated numbers of spores of high resistance, as only these expedients will furnish the required safety factors. Using just a few spores, or spores of low resistance (as is being done occasionally) is not germane to the problem posed.

Bacterial Spores as Test Material

It is rather generally accepted that the most reliable sterilizing agent is steam under pressure, when properly employed, as in an autoclave. Over a period of years, a very substantial literature has accumulated on the subject of steam sterilization, its advantages and limitations, its mode of killing microorganisms, etc. While most of the relevant theoretical and practical problems appear to have been settled, the same cannot be said about "chemical sterilization".

To begin with, until quite recently, there existed no standard method for determining sporicidal activity. As has been the case with other bacteriological test methods, the literature on chemical sterilization is replete with conflicting reports by different investigators. This is not particularly surprising in view of a variety of contributory factors, such as the indiscriminate use of non-representative species of spore-bearing microorganisms, the differences in the resistance of spores within individual species, the variations in the composition of culture and subculture media, also certain methodological shortcomings, e.g., failure to control sporostasis in subcultures.

The problem of elimination of sporostasis in the subculture is logically connected with that of the satisfaction of the exacting nutritional requirements of damaged but surviving spores. As has been shown so convincingly by Curran and Evans (5) in the case of spores exposed to the damaging action of certain physical and chemical factors, dramatic differences are observed in achieving survival by the use of different subculturing media. While in the case of "physical" damage the proper kind of enrichment of the medium should

be credited with promoting survival and subsequent germination of the spores, in the case of "chemical" damage it would not be feasible, in the absence of any special test results, to state whether in a particular instance the addition to the subculture medium of an "enriching" substance provides better nutritional conditions for the damaged organisms, or whether it acts in the direction of removing any inhibitory traces of the disinfectant transferred into the subculture through some form of interaction with it (e.g. chemical neutralization, complexing, sorption, etc.). Conceivably, both mechanisms may be involved. When, therefore, reference is made subsequently to the use of serum in the subculture as a means of controlling sporostasis, it should be understood that its possible effect in improving the nutritional quality of the medium is by no means discounted.

At various times, different chemical substances have been claimed to be possessed of sporicidal action. Disregarding those chemicals which would be too corrosive for practical purposes as instrument germicides, it seems that direct claims for sporicidal action *at room temperature* are being made today mostly on behalf of formaldehyde preparations although occasionally some iodophors and some quaternary ammonium compounds are being credited with sporicidal potency.

To dispose of the last claim first, reference should be made to a recent paper by Klarmann and Wright (6), who showed that the several quaternary ammonium compounds tested were not capable of killing the spores of *Cl. welchii, Cl. tetani, Cl. sporogenes* and *B. anthracis* even in 24 hours, thereby disproving the allegation as to the sporicidal potency of the "quats".

Is Formaldehyde Sporicidal Under Practical Conditions of use?

As to the action of formaldehyde on bacterial spores, there have been a number of developments in the recent past which bear importantly upon this matter.

Recently, Friedl (7) reported upon a collaborative study of a sporicidal test ("applicable for use with germicides to determine presence or absence of sporicidal activity and potential effectiveness in disinfecting against specified spore-forming bacteria") following the publication by Ortenzio, Stuart and Friedl (8) of a procedure for obtaining spores of the desired resistance and exposing them to the action of disinfectants. The method provides for the standardiza-

tion by means of "constant boiling hydrochloric acid" of the spores of two test organisms, viz. *Bacillus subtilis* and *Cl. sporogenes*, although it may be carried out also with *B. anthracis*, *Cl. tetani*, and other species of *Bacilli* or *Clostridia*.

With a formaldehyde germicide, "for which the manufacturer made sporicidal claims", Friedl reports the following results: Four collaborators found that the spores of *B. subtilis* were killed in thirty minutes, and two found this to occur within 10 minutes; however, all four agreed that the product did not kill the spores of *Cl. sporogenes* in two hours which was the longest period of exposure employed in this study.

These findings immediately indicate the grave risk involved in drawing conclusions concerning the sterilizing potency of a given preparation from the results obtained with the spores of a single species, such as *B. subtilis* whose pathogenicity, incidentally, is of a lesser relevance as compared with that of e.g., *Cl. tetani* or *Cl. perfringens*.

Extension of Tests on Spores

We were interested in following up these findings in three directions. Since the experimental data in Friedl's report do not go beyond a two-hour exposure period, it appeared desirable, for practical reasons, to extend the length of the observation to a full day or 8 hours. For the sake of expanding the validity of our conclusions, it was decided to add tests with two pathogenic spore-bearing microbes, viz. *Cl. tetani* and *Cl. perfringens*. And finally, we wanted to ascertain whether or not the factor of sporostasis in the subculture has been given its due attention, i.e., whether the trace quantity of the formaldehyde germicide adhering to the spores and to the spore carriers could be sufficient to affect the germination of the spores and proliferation of the vegetative forms in the subcultures.

With regard to the above points one and two, we employed the original method of Ortenzio, Stuart and Friedl. With regard to point three, we introduced a simple modification as follows: After completion of exposure of the spores to the germicide, the original procedure calls for subculturing in thioglycolate broth and incubation for 7 days, followed by transfer of the spore carrier to a second tube of thioglycolate broth and incubation for 7 more days; our modification of this procedure consists in leaving the spore carriers

in the first subculture tube for only 10 minutes, and transferring (after thorough agitation) to a second tube of thioglycolate broth containing 10 per cent of horse serum.

As to the rationale of this modification, it was assumed that a 10-minute exposure to broth in the first subculture tube was sufficient to take care of the dilution factor. Moreover, it was observed that even with the very long sojourn of the spores in the first transfer tube, as prescribed by the original method, sporostasis was still in evidence upon transfer to the second subculture tube; however a small proportion of horse blood serum was found to be capable of suppressing this stasis. It is held that there can be no question as to the propriety of using biological material, such as serum, in a test of this kind since objects presumably sterilized with formaldehyde eventually come in contact with tissue fluids of a related chemical character which would, therefore, have the same "neutralizing" effect upon the inhibitory traces of formaldehyde adhering to any spores, as exhibited by the serum employed in our experiments (9, 10). (This is also why supplementary experiments are now being planned to determine the corresponding effect of blood as well as of higher serum concentrations.)

The following table I indicates the resistance to constant boiling hydrochloric acid of the four spore-bearing microorganisms employed, as established by the original method of Ortenzio, Stuart and Friedl (I), and by our modification (II) respectively.

TABLE I

Resistance of Spores to "Constant Boiling Hydrochloric Acid"
Time of Exposure (In Hours)

Spores of:	Method	1	2	4	6	8
B. subtilis	I	+	+	+	—	—*
	II	+	+	+	—	—
Cl. sporogenes	I	+	+	+	—	—
	II	+	+	+	±	—
Cl. perfringens	II	+	+	+	+	±
Cl. tetani	II	+	+	+	+	+

It appears that the spores of *Cl. sporogenes* and *B. subtilis* display substantially the same resistance to constant boiling hydrochloric

* All data given in this and in the following tables are based on five to ten replicate tests intended to take care of fluctuation of results.

acid, regardless of whether the original or the modified method of subculturing is employed; this finding contributes to the validation of the admissibility of the modification introduced.

Table II gives the results obtained with the formaldehyde germicide by the two methods referred to above.

TABLE II

RESISTANCE OF SPORES TO FORMALDEHYDE GERMICIDE
TIME OF EXPOSURE (IN HOURS)

Spores of:	Method	1	2	4	6	8
B. subtilis	I	—	—	—	—	—
	II	+	+	+	—	—
Cl. sporogenes	I		—	—	—	—
	II		+	+	+	±
Cl. perfringens	I		+	±	—	—
	II		+	+	+	±
Cl. tetani	I		+	±	—	—
	II		+	+	±	±

It will be seen that the expedient of controlling the inhibitory effect of formaldehyde by means of serum in the subtransfer tubes changes the picture rather materially. Thus the spores of *Cl. sporogenes* which appear to have been killed after a 2 hour exposure to the formaldehyde germicide, are not always killed even in 8 hours. A similar situation is encountered with respect to the spores of *Cl. tetani* and *Cl. perfringens* while with *B. subtilis* spores, sterilization is achieved evidently only after 6 hours rather than after 1 hour of exposure.

Of course, the extension of the exposure period to 8 hours or beyond raises the problem of practicality of any such procedure, from the point of view of sterilization technique as practiced by particular hospitals. Moreover, it would seem entirely logical to argue that the test method described tends to oversimplify the conditions of practice, in that no account is taken of certain interfering factors such as the difficult accessibility of parts of some hinged of hollow instruments, the protective entrapment of spores by formaldehyde coagulated albuminous matter (left behind after incomplete mechanical cleaning), etc. which could retard the process of sterilization to a minor or major degree.

By way of furnishing confirmatory data, Table III presents the results obtained with an 8 per cent aqueous solution of formaldehyde

prepared in the laboratory. The technique employed here provides for the control of sporostasis by means of serum in the subtransfers (Method II).

TABLE III

RESISTANCE OF SPORES TO 8 PER CENT FORMALDEHYDE (AQUEOUS)
TIME OF EXPOSURE (IN HOURS)

Spores of:	Method	1	2	4	6	8
B. subtilis	II	±	—	—	—	—
Cl. sporogenes	II	+	+	+	+	±
Cl. perfringens	II		+	+	+	+
Cl. tetani	II		+	+	+	±

This table shows that an 8 per cent aqueous formaldehyde solution is not capable of killing the spores of any of the three *Clostridia* in 8 hours, in every case. *B. subtilis* spores are killed in 2 hours, a finding which points to their lower resistance to formaldehyde, as compared with that of the *Clostridia*.*

By way of supplementary information obtained by us, *Cl. sporogenes* spores were not killed by a 5 per cent solution of phenol, nor by a 3 per cent solution of a commercial iodophor product (with 1.6 per cent of available iodine) in 8 hours; they were killed by the undiluted iodophor within this period of time in some, but not in all cases. Undiluted Strong Iodine Solution U. S. P., however was sporicidal in 2 hours (or possibly less) while upon dilution with an equal volume of water it was active only in 8, but not in 4 hours. Neither a 2 nor a 10 per cent solution of a commercial hypochlorite preparation (with 5.2 per cent of available chlorine) killed these spores in 8 hours.

Sterilization by Boiling in the Presence of Disinfectants

As has been pointed out before, autoclaving properly performed, may be relied upon to produce true sterilization, including destruction of resistant spores. Nevertheless, boiling of instruments is still being practiced extensively, but without apparent realization of the shortcomings of this procedure with regard to sporicidal action. Ex-

* The addition to alcoholic formaldehyde of hexachlorophene should not be expected to change the sporicidal performance to any significant degree, since hexachlorophene is mostly bacteriostatic in character, and since its anti-bacterial action is subject to reversal by tissue fluids (10).

periments performed in our laboratory show that spores of *Cl. sporogenes* standardized for resistance against constant boiling hydrochloric acid, withstand boiling water (100° C.) for 2 to 3 hours. It appeared tempting, therefore, to ascertain the effect of combining the action of boiling water with that of antibacterial agents which, from a practical point of view, could be considered to be of practical interest for such a combination.

The modified testing procedure described before was used here, except that the medication tubes were held at 98° C. by means of a glycerin bath. The results are given in the following Table IV. (This table gives the active ingredients of the disinfectants tested, also the concentrations in terms of the complete formulations. The results are representative of 5 to 10 individual tests in every instance.)

TABLE IV

RESISTANCE OF CL. SPOROGENES SPORES TO BOILING DISINFECTANT SOLUTIONS
TIME OF EXPOSURE (IN MINUTES)

Disinfectants (Active Ingredients)	5	10	15	20	30
Phenol, 2%	+	+	+	±	—
Cresol (N. F.), 2%	+	+	±	—	—
o-Phenylphenol, 2%	±	±	—	—	—
o-Phenylphenol and p-tert. Amylphenol, 1%	±	±	—	—	—
o-Phenylphenol and p-tert. Amylphenol, 2%	±	±	—	—	—

It appears that with the aid of certain germicidal solutions heated close to the boiling point, it is possible to kill the spores of *Cl. sporogenes* within a practical period of time. Although 2 per cent solutions of phenol and of Cresol Compound N. F. produce this effect in 30 and 20 minutes respectively their pungent odor is intensified by heating to such an extent as to make it decidedly objectionable. This criticism does not apply to the synthetic phenolic disinfectants tested * whose active ingredients exhibit a low degree of volatility thereby creating no significant odor problem; their 2 per cent solutions kill the spores in 15 minutes.

* The two synthetic phenolics listed in Tables IV and V, with o-phenylphenol as their main active ingredient, contain potassium ricinoleate as their primary emulsifying agent. They are known under the trade names "O-syl" and "Amphyl", respectively.

A benzalkonium chloride solution (1:1000) heated to the boiling point killed the spores in 30 minutes, but produced a rusting effect on the blades. A 2 per cent iodophor solution damaged the blades within the first two minutes. This is in contrast to the 2 per cent solutions of the synthetic phonolics which at boiling temperature exhibit anti-rusting action.

As has been shown in the preceding paragraphs, the elimination from surgical instruments of a significant spore contamination cannot be entrusted, with complete safety, to a chemical germicide acting at room temperature. While sterilization by steam under pressure is a satisfactory sterilizing procedure, it appears now that contact with a suitable dilute disinfectant solution heated to near boiling furnishes another method of sterilization, comparable in its effectiveness as well as in its practicality to autoclaving.

Cold Disinfection (Tuberculocidal)

But what about instruments which cannot be exposed to heat in any form? In view of the existing situation, it is held that such instruments should be disinfected with the aid of non-specific chemical agents, i.e., those possessed of demonstrable microbicidal potency with respect to the different pathogens of surgical and related significance (except resistant bacterial spores). The bactericidal spectrum of an acceptable chemical instrument disinfectant should include tuberculocidal potency in view of the fact that the objects in question comprise, among other things, endoscopic instruments (e.g. bronchoscopes) which while in use, may come in contact with tubercle bacilli in the presence of the characteristic sputum interposing a major obstacle to the action of the disinfectant upon the bacterial cells. Similar considerations apply to the disinfection of clinical thermometers which, too, cannot be subjected to heat treatment.

A first approximation to the screening of suitable chemical germicides for use with instruments is furnished by the "Use-Dilution Confirmation Test" which now enjoys a quasi-official status having originated with a regulatory government agency (11). Basically, the test procedure calls for exposure to the action of disinfectants of two test organisms deposited on carriers, viz. *Salmonella choleraesuis* and *Micrococcus pyogenes* var. *aureus*. (The former is presumed to serve as representative of the enteric, the latter as that of the

pyogenic variety of pathogens). While reference should be made to the original publication for the underlying philosophy as well as for the details of this test, it should be stated here that the maximum dilution of a germicide recommended for surgical use which is found to be effective under the experimental conditions of the test, is presumed to be capable of disinfecting scalpel blades contaminated with a variety of pyogenic microorganisms such as *M. pyogenes* var. *aureus* and *albus*, also *Streptococcus pyogenes, fecalis* and *agalactiae*, even in the presence of dried blood. Incidentally where a given product, or its recommended use dilution does not satisfy the performance requirements of this test, a misbranding charge may be made against the purveyor of such a product, under the pertinent provisions of the Federal Insecticide, Fungicide and Rodenticide Act.

While, therefore, the information furnished by the results of this test is of the greatest interest in connection with the performance of a given preparation represented as an instrument germicide, it is also incomplete with reference to the important question as to its capacity for tuberculocidal action.

Frobisher, Sommermeyer and Blackwell (12) published a method of determining tuberculocidal action which embodies a number of features suitable to make the procedure simulate the conditions of practice; probably the most important of these is the use of fresh, unbroken sputum from active cases of pulmonary tuberculosis which did not receive any chemotherapy, thereby eliminating the question of any attenuation of the microorganism. While the several original papers should be consulted with regard to details of the technique employed and of the results obtained, it should be mentioned here that of the materials tested, a solution of 0.5 to 1 per cent of iodine in 70 per cent ethyl or isopropyl alcohol produced a satisfactory tuberculocidal disinfection of contaminated thermometers within 10 minutes, provided that they were subjected to prior cleaning with an alcoholic soap solution of specified composition. By contrast, neither formaldehyde (in 4 or 10 per cent solutions) nor quaternary ammonium compounds (in 0.1 per cent aqueous solutions) produced the desired results; the latter, when used in the form of alcoholic tinctures appeared to perform satisfactorily, although unfortunately, no attempt was made here to ascertain the presence or absence of bacteriostasis, thereby leaving the question open as to the fitness of the tinctures to serve as thermometer disinfectants.

The latter consideration relates directly to the previous work of Smith, *et al.* (13, 14) who found quaternary ammonium compounds to be of no value as disinfectants for use in tuberculosis hygiene, along with a number of other surfactants of anionic and non-ionic character.

We carried out a number of experiments upon undigested sputum deposited on thermometer pieces using both the original method of Frobisher, Sommermeyer and Blackwell, and also a slightly modified procedure in which Dubos agar was substituted for the Lowenstein slants, as this expedient assures the dilution below the bacteriostatic level of the traces of disinfectant, particularly of the quaternary ammonium variety, transferred on the carriers from the medication vessels to the subcultures (as verified experimentally by reinoculation and examination for growth, following incubation for the required period of time; it was also confirmed by means of control tests that this modification had no effect upon the viability of the test organism as compared with the original procedure). The results are given in Table V (which states only the active ingredients of the disinfectants tested, also the concentrations in terms of the complete formulations).

TABLE V

DISINFECTION OF THERMOMETERS CONTAMINATED WITH TUBERCULAR SPUTUM
IMMERSION PERIOD 10 MINUTES

Disinfectant (Active Ingredients)	Dilution	Effect Per Thermometer 1	2	3	4	5
o-Phenylphenol	1:50	0	0	0	0	0
	1:100	0	0	0	0	C
o-Phenylphenol and p-tert. Amylphenol	1:100	0	0	0	0	0
Iodophor (1.6% av. I)	1:50	+	+	+	0	C
	1:100	+	+	+	0	C
	1:213	+	+	+	C	C
	1:320	+	+	+	C	C
Iodophor (0.45% av. I)	1:10	+	+	+	0	0
	1:50	+	C	C	C	C
	1:100	+	+	C	C	C
Water Controls		+	+	C	C	C

0 No colonies of *M. tuberculosis* found.

C Plate too heavily contaminated to read results.

\+ Colonies of *M. tuberculosis.*

This table shows that thermometer pieces contaminated with tubercular sputum (containing over 27,000 mycobacteria per ml.)

can be disinfected within 10 minutes by immersion in dilute solutions of the two synthetic phenolic germicides tested (2 and 1 per cent respectively).

With one iodophor product (containing 1.6 per cent of available iodine) disinfection could not be effected using dilutions ranging from 0.3 to 2 per cent (i.e., 50 to 320 ppm of iodine). With another iodophor (containing 0.45 per cent of available iodine), no reliable disinfection could be achieved with a 10 per cent solution (i.e., with 450 ppm of iodine). As to the performance of other materials under these or similar conditions reference should be made to the papers by Frobisher *et al.* mentioned above.*

Experimental Part

Modified Procedure for Ascertaining Sporicidal Action. This procedure is based upon the method of Ortenzio, Stuart and Friedl (8).

Cultures of *Cl. sporogenes* were grown for three days at 37° C. in egg-meat-soil-extract media. Black silk suture loops (number 3 surgical silk) were placed in the culture for 15 minutes. They were then withdrawn and dried on filter paper for 24 hours. The resistance of the spores to constant boiling hydrochloric acid was now determined, by placing some loops in tubes containing it, and held at 20° C. At stated time intervals the loops were withdrawn and transferred to an alkaline thioglycolate medium (National Institutes of Health). The remainder of the loops were allowed to dry for 7 days at which time they were placed in the solutions of the disinfectants under test, at 20° C. The loops were removed at stated time intervals and transferred to thioglycolate broth. After 10 minutes of standing and thorough shaking, they were retransferred to second tubes of thioglycolate broth containing 10% of horse serum. All tubes were incubated for three weeks, and a few were observed for two months. Generally speaking, no changes in the results were noted after five days' incubation.

* Of course, it is not suggested that preliminary, mechanical cleansing of sputum contaminated instruments should be dispensed with; obviously, a thick coating of active sputum settling, e.g., in the crevice of a hinged instrument or in the lumen of a tubular appliance should not be expected to yield to penetration and disinfection in so short a period of time. The experiments reported are intended to be illustrative rather than to represent any recommended technique of disinfection.

Modified Procedure for Ascertaining Tuberculocidal Action. This procedure is based upon the method of Frobisher, Sommermeyer, and Blackwell (12).

The test objects were pieces of thermometers, 2½ inches long and sealed on the broken ends. The sputum used in the test was a pooled sputum from three tubercular patients who had received no chemotherapy for one year. By plate counts there were 27,100 bacilli per ml.

The medium used was a modification of Dubos agar.

2 gms. Sheffield hydrolyzed casein
6.3 gms. dibasic sodium phosphate
1 gm. monobasic potassium phosphate
1.5 bms. sodium citrate
2 gms. Difco yeast extract
15 gms. agar

pH 7.2

This basic medium is enriched by the addition of 10% Fraction V extract.

25 gms. bovine albumin powder fraction V (Armour Labs.)
500 ml. warm physiological salt solution
0.3 ml. oleic acid in 25 ml. N/20 NaOH
25 gms. glucose

Sufficient penicillin was added to the above extract so that each 2 ml. (enough for 1 petri dish) contained 1500 units of penicillin. The extract was filtered to obtain sterility.

The unbroken sputum was placed in a deep petri dish and the thermometer pieces added. They were removed with forceps, placed on stainless steel wire mesh and allowed to dry for 30 minutes. Some were more heavily contaminated than others and were not completely dry at the end of the thirty minute interval. They were then transferred to 20 ml. of disinfectant (enough to cover completely) in a water bath at 20° C. They were left in the disinfectant solution for 10 minutes and then transferred to a petri dish containing 2 ml. of Fraction V extract. Twenty ml. of melted agar at 45° C. was added, the plates were well agitated and then incubated at 37° C. for 10 weeks in a moist chamber. In the plates showing *M. tuberculosis* growth, the colonies were apt to be grouped around the thermometer piece. Satisfactory counts could not be made. Therefore,

the plates are designated either +, or 0 for *M. tuberculosis*. Some colonies from each plate were identified by acid fast staining. Five thermometer pieces were tested for each dilution of disinfectant. Control thermometer pieces were dipped in water only. Ten were tested as controls as it was thought that many would show gross contaminations. A sodium hydroxide digest was made of the sputum, and plates made of dilutions of this to determine the number of *M. tuberculosis* per ml. of the sputum sample.

Elimination of Bacteriostasis: Previous tests made with the several disinfectants tested (Table V) had shown that amounts of disinfectant ten times greater than those transferred into the subcultures produced only slight bacteriostasis, and that the amounts occurring in the transfers produced no bacteriostasis.

Summary

The occurrence of bacterial spores upon surgical instruments may be of major or minor significance depending upon the physiological or pathological matter with which they may come in contact. By the same token, sterilization involving assured elimination of spores may be essential in some instances while disinfection, involving non-specific microbicidal action but not necessarily sporicidal effectiveness, may be sufficient in others.

Steam under pressure properly applied is known to be the sterilizing agent *par excellence* for instruments and appliances. It has been found that the same results, particularly with respect to resistant bacterial spores, may be produced in a short period of time by exposure to boiling dilute aqueous solutions of certain disinfectants, selected preferably from the class of synthetic phenolics of low volatility with water vapors.

Formaldehyde preparations acting at room temperature have not been found to be reliably effective against resistant bacterial spores even after 8 hours of contact when tested under experimental conditions which provide for the control of sporostasis in subcultures. There may be some question as to the practicality of procedures involving exposure periods of such, or rather, of greater lengths.

With reference to the cold disinfection of instruments it is postulated that tuberculocidal action should be part of the non-specific bactericidal spectrum of a disinfectant recommended for use on objects which cannot be subjected to heat treatment in any form; the same requirement should apply to disinfectants for clinical thermometers.

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COMPARATIVE PALISADE NUMBERS AS A MEANS OF MICROSCOPICALLY SEPARATING AND IDENTIFYING PEPPERMINT AND SPEARMINT LEAVES

By Harold I. Silverman * and Marin S. Dunn **

THE authors have found the method outlined in this paper satisfactory for accurately identifying by the use of the microscope the crude drugs, peppermint and spearmint whether alone or in admixture, whole or powdered.

The method has been employed in our laboratories thirty-five times on authenticated samples of each of the above-mentioned drugs, and it has proved itself to be unfailingly successful. Both larger and smaller leaves have been used from material collected at various times of the year from different locations in the United States. A few imported samples were also included. Initially, the work was confined to samples of whole leaves from herbariums and from growing material. Then powdered samples of ten of the above were used, the method proving successful with powders passing thru sieves up to and including 120 mesh.

It was also found from prepared samples that the presence of these drugs could be recognized when powdered and in admixture.

The necessary materials are a compound microscope having a 10X ocular and 10X and 43X objectives and a Howard Micrometer Disc (Fig. 1) which fits inside the 10X ocular. A focusing microscope illuminating lamp is desirable, but not absolutely necessary. The micrometer disc ruling should be standardized. In our work the ocular net measured 54 microns per side on each small rectangle when using the 43X objective.

The method employed involves counting the number of palisade cells, seen in surface view, which are confined to a definite area of a cleared leaf. In our case, the area was that of a single rectangle of a Howard Micrometer Disc and the counting was done using the 43X

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objective, the 10X objective being employed for rapid scanning of the field. In using this method, magnification and length of sides of the ocular net should always be reported. We have found that a comparison of palisade numbers thus obtained in the case of peppermint and spearmint is quite an easy and valuable means of identifying the two species.

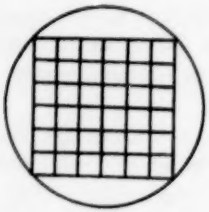

FIG. 1
ILLUSTRATION OF RETICULE
Use the centrally-located rectangles to count the confined palisade cells.

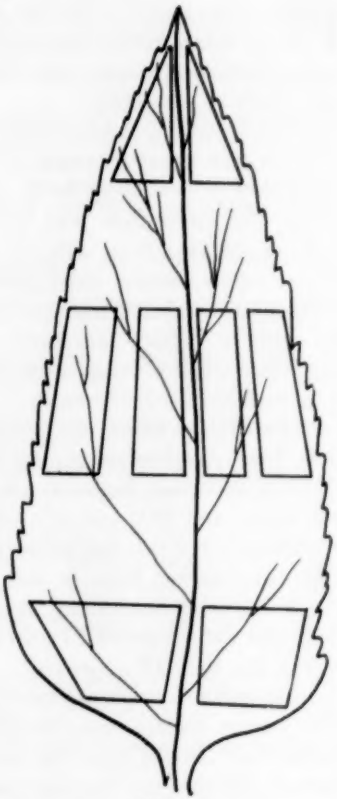

FIG. 2
ILLUSTRATION OF LEAF APPROXIMATELY 2 TIMES ACTUAL SIZE
Each block represents an area where 2 separate counts are made.

Using an entire leaf, the procedure is as follows: The leaf is first cleared in a solution of chloral hydrate (25 gm. of chloral hydrate per 10 ml. of water) either by standing in this solution for several days or by the use of gentle heat (below boiling). The latter treatment usually renders the material ready for examination within one or two hours at most. The leaf is then placed with its upper surface uppermost on a slide and mounted in Berlese mountant under a cover glass of a size suitable to cover the entire leaf. The leaf is then examined under the 10X objective to determine whether the

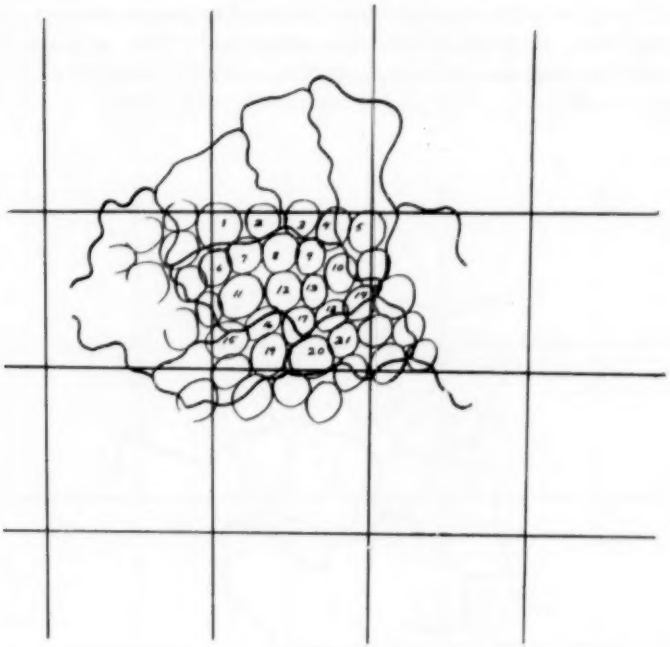

FIG. 3

SPEARMINT

Typical appearance of leaf as viewed through reticule (portion of) approximate magnification 235X.

The count is done by counting all palisade cells that are 50% or more enclosed by the boundaries of the counting area,—in the above illustration all these have been numbered. In case of doubt do not count the palisade cell, counting only those which appear to be definitely 50% or more within the boundaries.

epidermal cells are sufficiently transparent so that focusing upon the underlying palisade cells can be readily and clearly accomplished. If the latter are easily seen, the 43X objective is then used to count the number of palisade cells that appear within the confines of a single centrally-located rectangle of the reticule.

In our counts of entire leaves, four distinct areas were chosen: the tip, sides (a point halfway between the tip and base at margin), midrib (a point halfway between tip and sides), and base of the lamina (Fig. 2). This method was followed in order to determine the effect of position upon count. Our studies do not indicate troublesome variation in the average values obtained by counts whether done on tip, base, sides or midrib of a single leaf. For each position chosen, four counts were made, so that for each leaf there were sixteen counts in all.

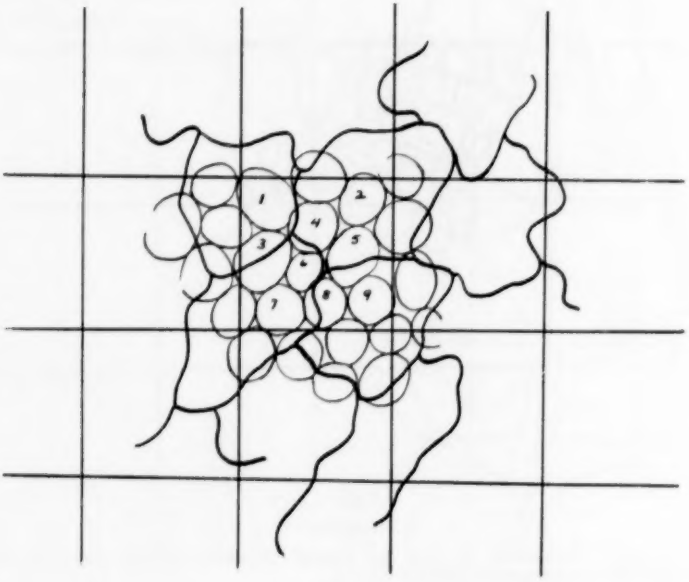

FIG. 4
PEPPERMINT

Typical appearance of leaf as viewed through reticule (portion of) approximate magnification 235X.

MENTHA SPICATA L. had a range of 12-33 and an average of 20 for its palisade number (Fig. 3).

MENTHA PIPERITA L. had a range of 5-13 and an average of 9 for its palisade number (Fig. 4).

The above palisade numbers are each based on over six hundred determinations (thirty five samples times sixteen determinations per sample plus powder determinations).

Since the average values of the palisade counts of the two species, are quite different, one can see that by examining either an entire leaf or a portion thereof, as in the case of a powder (powders are cleared in the same way), as long as the palisade cells can be counted, there will be sufficient evidence for the identification of a sample.

The method appears to offer promise with finer powders by employing an ocular net with smaller rectangles.

THE FORMULATION OF AN ORAL SUSPENSION OF DIPHENYLHYDANTOIN

By Clyde F. Wiegand,* C. Lee Huyck **

THE object of this project is to develop an acceptable suspension of diphenylhydantoin. To indicate the stability of four proposed formulas, and to note the effect of temperature and time; the pH, assay results, viscosity, and sedimentation rate were measured over a period of one month.

Gastric distress and nausea may result from the oral administration of the sodium salt of diphenylhydantoin because of the alkalinity of the drug (1). Diphenylhydantoin ($C_{15}H_{12}N_2O_2$) would be indicated in lieu of its sodium salt when formulating a suspension. As the solubility of this antiepileptic drug in alcohol is only one gram in about sixty ml. (2), an attempt at formulating an alcoholic solution of the drug would be impractical.

The four suspensions were prepared as follows :

(A)

Diphenylhydantoin [1]	5 grams
Sodium Alginate (Kelgin) 1.5%	375 ml.
Glycerin	10 ml.
Oil of Anise	0.4 ml.
Propylparaben	0.5 gram
Ethyl Alcohol 95%	6 ml.
Syrpalta [2] Q.S. ad	500 ml.

The sodium alginate was suspended in the glycerin. Hot distilled water was then added to volume, with agitation. The resulting colloidal dispersion was then used to suspend the drug in the form of #100 mesh powder. The volatile oil in alcoholic solution and propylparaben in glycerin solution were then added. This mixture

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1. Courtesy Parke Davis & Co., Detroit, Mich.
2. Syrpalta (Emerson Co., Dallas, Tex.).

was then diluted to final volume with Syrpalta and the final product was then agitated in an electric mixer until a uniform dispersion was accomplished.

Syrpalta was employed in two capacities: First as a flavoring vehicle, and second as a buffer. This artificially flavored grape syrup was found to be satisfactory for masking the taste of the drug, especially when used in combination with a suitable volatile oil. The pH ranges 4.8 to 6.8 of the four formulas appeared desirable as evidenced from the assay results reported in the table. The pH of a commercially marketed suspension of diphenylhydantoin was found to be 4.5.

(B)

Diphenylhydantoin	5 grams
Methyl Cellulose, 4000 cps., 2%	375 ml.
Oil of Cinnamon	0.5 ml.
Ethyl Alcohol 95%	1 ml.
Sodium Benzoate	0.5 gram
Distilled Water	1 ml.
Syrpalta Q.S. ad	500 ml.

Methyl Cellulose 4000 cps. was mixed with 200 ml. of distilled water which had been brought to boiling. This mixture was allowed to macerate for thirty minutes. The remaining 175 ml. of distilled water was then added gradually with agitation. The resulting colloidal dispersion was then used to suspend the drug in the form of #100 mesh powder. The volatile oil in alcoholic solution and the sodium benzoate in aqueous solution were then added. This mixture was then diluted to final volume with Syrpalta and the final product was then agitated in an electric mixer until a uniform dispersion resulted.

(C)

Diphenylhydantoin	5 grams
Sodium Carboxymethylcellulose 2% (70 low)	375 ml.
Oil of Fennel	0.4 ml.
Ethyl Alcohol 95%	1 ml.
Sodium Benzoate	0.5 gram
Distilled Water	1 ml.
Syrpalta Q.S. ad	500 ml.

Sodium carboxymethylcellulose was mixed with about 20 ml. of distilled water to produce a fine paste. This was then diluted to volume with distilled water with agitation. The resulting colloidal dispersion was then used to suspend the drug in the form of #100 mesh powder. The volatile oil in alcoholic solution, and the sodium benzoate in aqueous solution were then added. This mixture was then diluted to final volume with Syrpalta and the final product was then agitated in an electric mixer until a uniform dispersion resulted.

(D)

Diphenylhydantoin	5 grams
Oil of Anise	0.4 ml.
Ethyl Alcohol 95%	1 ml.
Syrpalta Q.S. ad	500 ml.

Diphenylhydantoin in the form of #100 mesh powder was mixed with 20 ml. of Syrpalta, resulting in a fine paste. This was diluted, gradually with agitation to volume with the balance of the Syrpalta. The volatile oil in alcoholic solution was then added. The resulting suspension was agitated in an electric mixer to produce a uniform dispersion.

Stability tests for the four formulae were conducted at room temperature, at 40° C., and at 10° C. The effect of time and temperature on pH, viscosity and assay results are reported on the data sheet.

Experimental Data Sheet

Abbreviations RT, 40 and 10 added to formula letter indicate product stored at room temperature, at 40° C. and at 10° C. respectively.

Formula	*Assay (mg. Diphenyl-hydantoin)*	*Viscosity (cps.)*	*pH*	*Date*
A	285	2400	6.8	7- 2-55
B	—	250	4.8	7- 2-55
C	310	800	6.8	7- 2-55
D	300	450	5.5	7- 2-55
A-RT	285	2400	6.8	7- 9-55
A-40	285	2400	6.8	7- 9-55
A-10	285	2400	6.8	7- 9-55
B-RT	—	250	4.8	7- 9-55

Formula	*Assay (mg. Diphenyl-hydantoin)*	*Viscosity (cps.)*	*pH*	*Date*
B-40	—	250	4.8	7- 9-55
B-10	—	250	4.8	7- 9-55
C-RT	300	800	6.8	7- 9-55
C-40	315	800	6.5	7- 9-55
C-10	310	800	6.8	7- 9-55
D-RT	310	450	5.5	7- 9-55
D-40	300	440	5.5	7- 9-55
D-10	290	450	5.5	7- 9-55
A-RT	290	2400	6.7	7-16-55
A-40	285	2450	6.4	7-16-55
A-10	285	2400	6.8	7-16-55
B-RT	—	250	4.8	7-16-55
B-40	—	240	4.8	7-16-55
B-10	—	250	4.8	7-16-55
C-RT	310	800	6.8	7-16-55
C-40	310	800	6.3	7-16-55
C-10	310	800	6.8	7-16-55
D-RT	300	450	5.5	7-16-55
D-40	290	450	5.3	7-16-55
D-10	300	450	5.5	7-16-55
A-RT	285	2450	6.7	7-23-55
A-40	273	2400	6.4	7-23-55
A-10	285	2400	6.8	7-23-55
B-RT	307	240	4.8	7-23-55
B-40	292	250	4.8	7-23-55
B-10	310	250	4.8	7-23-55
C-RT	310	800	6.8	7-23-55
C-40	300	800	6.3	7-23-55
C-10	310	800	6.8	7-23-55
D-RT	300	450	5.4	7-23-55
D-40	300	450	5.3	7-23-55
D-10	300	450	5.5	7-23-55
A-RT	285	2400	6.7	7-30-55
A-40	285	2450	6.4	7-30-55
A-10	275	2450	6.8	7-30-55
B-RT	305	250	4.8	7-30-55
B-40	290	250	4.8	7-30-55

Formula	*Assay (mg. Diphenyl-hydantoin)*	*Viscosity (cps.)*	*pH*	*Date*
B-10	315	250	4.8	7-30-55
C-RT	310	800	6.8	7-30-55
C-40	300	800	6.3	7-30-55
C-10	295	800	6.8	7-30-55
D-RT	310	450	5.3	7-30-55
D-40	300	450	5.3	7-30-55
D-10	310	450	5.5	7-30-55

pH readings recorded represent two consecutive determinations at 25° C. on a Beckman pH Meter.

Viscosity determinations reported on data sheet represent the average of three determinations at 25° C., using a Brookfield Synchro-Lectric Viscometer, Model "R. V. F." Determinations were made using various needles and varying rates of shear.

Assay results reported in mg. of diphenylhydantoin per 30 ml. of formula represent the average of two gravimetric determinations. The assay procedure is a modification of the U. S. P. XIV Assay for diphenylhydantoin sodium (3):

> Measure accurately 30 ml. of an aliquot portion of the suspension, representing 300 mg. of diphenylhydantoin, and transfer it to a separatory funnel. Add 50 ml. of distilled water, and extract it with 100 ml. of absolute ether, followed by four successive portions of 25 ml. of absolute ether. Evaporate the combined ether extracts and dry the residue at 105° F. for four hours, then weigh accurately in a previously tared beaker.

This modified assay procedure was found to be satisfactory for formulae (A), (C), and (D). However, this procedure was totally unsatisfactory for the assay of formula (B) due to an apparent solubilization or partial emulsification of ether by the methyl cellulose. This was evidenced by the lack of a separation between the ethereal and aqueous phases during the extraction procedures. An effort was made to find another organic solvent in which the diphenylhydantoin is sufficiently soluble, but immiscible with water. Chloroform was found to be satisfactory for this purpose, and was, therefore, employed in lieu of ether in the assay procedure previously described.

Upon centrifuging, sedimentation of formulae (A), (B), and (C) was less than 0.1 ml. per 3.0 ml. of suspension. Formula (D) exhibited virtually no sedimentation upon centrifuging. This fact was considered as indicative of the suspending powder of Syrpalta, since none of the synthetic gums were utilized in this formula. The viscosity of Syrpalta is 450 cps.

Observations

Judging from assay results reported, all four formulas are reasonably stable for the one month period of time in which the tests were conducted. There was no apparent change in viscosity in any of the formulas. The largest decrease in pH was 0.5 for formula (C) after subjection to 40° C. for a period of three weeks. Formula (B) was found to foam excessively upon shaking. Uniform dosage measurement by the patient would be somewhat hindered. Partial gelatinization was observed in formula (B) after subjection to 40° C. for six days, but the product returned to its original fluidity upon cooling. Redispersion of pronounced sedimentation in all refrigerated products was easily accomplished upon agitation and slight heating. Formulas (A) and (C) were the most elegant of the four in appearance. Flavoring was satisfactory in all products, since the taste of the Syrpalta and volatile oil predominated.

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SELECTED ABSTRACTS

The Reversal of Certain Concomitants of Aging With Vitamin Therapy. Kaufman, W. *J. Am. Geriat. Soc. 3:927* (1955). Concomitant with aging there are a series of effects such as reduced joint flexibility, reduced muscular strength, impaired coordination and sense of balance, mental changes, and increased capillary fragility. Over a period of some twelve years the author studied the effect of large doses of niacinamide with or without other vitamins upon these symptoms of aging.

A group of 663 persons accepted treatment with niacinamide in doses ranging from 900 to 4,000 mg. a day, depending upon the severity of joint dysfunction. A significant improvement in joint mobility was observed without exception and irrespective of age. This improvement was maintained for as long as treatment was continued. This action of niacinamide was not enhanced or retarded by the addition of up to 4,000 mg. of ascorbic acid, 54 mg. riboflavin, 27 mg. thiamine, 54 mg. pyridoxine, 54 mg. calcium pantothenate, up to 10,000 units of vitamin A, up to 9,000 units of vitamin D or 100 micrograms of vitamin B_{12}.

Soon after niacinamide therapy was begun, many patients, particularly those in the older age groups, noticed an improvement in their strength and exercise tolerance. Some patients who did not respond to niacinamide alone did respond upon the addition of thiamine and riboflavin or choline dihydrogen citrate. About 30 per cent of the patients showed no improvement in thier muscle strength. The sense of balance also improved in some patients, but not in all. The addition of vitamin B_1, B_6, and B_{12} brought improvement to some patients who did not respond to niacinamide alone. A similar improvement was observed in some of the patients who were mentally depressed or were hyperkinetic.

Niacinamide had no effect upon the tendency for increased capillary fragility to occur in aging persons. However, the daily administration of 1,500 to 2,000 mg. of ascorbic acid did reduce capillary fragility for as long as it was administered. Thus it would appear that some of the concomitant symptoms associated with aging can be reversed to a significant degree.

The Effect of Insulinase-Inhibitor on the Action of Insulin. Mirsky, I. A., and Perisutti, G. *Science 122:*559 (1955). It has been demonstrated that exogenous insulin is destroyed by the intact animal as well as by homogenates, extracts, and slices of liver and other tissues. This destruction is apparently due to an enzyme system which has been named, insulinase. A nonprotein liver fraction has been found which inhibits the *in vitro* inactivation of insulin by homogenates and slices of liver. The active component of this liver fraction has been termed, insulinase-inhibitor.

In studies with rats and with rabbits, the authors found that the intraperitoneal injection of insulin (in rats) or intravenous injection (in rabbits) resulted in a potentiation of the hypoglycemic effect when a subcutaneous injection of the insulinase-inhibitor preparation had been given an hour previously. This finding indicates that the insulinase-inhibitor is effective in increasing the biological activity of insulin in rats and rabbits. The insulinase-inhibitor preparation, however, proved to be very toxic to rabbits although not to rats.

A New Drug for the Relief of Dysmenorrhea. Jones, S. S. *Northwest Med. 54:*1253 (1955). A uterine relaxing factor, lututrin, obtained from the corpus luteum of sow ovaries and standardized for potency in terms of units of activity on the guinea pig uterus, was used by the author in the treatment of dysmenorrhea in 40 cases. The usual dose is 2000 to 4000 units, as 1000 unit tablets, when the cramps first begin. Following this, 1000 to 3000 units are taken every 3 to 4 hours if necessary as long as needed to keep the patient free from cramps.

Of the 40 patients treated, 57.5 per cent had all symptoms relieved, 30 per cent had a marked abatement of the cramps and the patient was able to go about her work, and the others had only slight or no relief.

The author pointed out that adequate dosage is necessary and that he feels that 4000 units is probably the desirable initial dose. He stated that this therapy gave better results than any other type of treatment which he had ever used. No signs of toxicity were evident.

A Combination of Polymyxin B With Oxytetracycline for Topical Therapy. Carsley, E. H. *Antibiot. Med. 1:567* (1955). The addition of polymyxin B to an ointment containing oxytetracycline was an attempt to increase the antibacterial spectrum to include *Pseudomonas aeruginosa,* a frequent secondary invader in skin infections. This seemed to be a natural development in an attempt to provide a more complete topical preparation for the treatment of pyogenic skin lesions.

The ointment contained 30 mg. of oxytetracycline and 1 mg. of polymyxin B per Gm. of petrolatum ointment base. It was used in the treatment of 75 patients having a variety of skin disorders, mostly pyodermas.

In 21 patients with acute impetigo, improvement was observed in 24 hours and clearing was complete in 3 to 7 days. The author stated that these results were the best ever obtained with any antibiotic preparation. All of 12 patients with sycosis barbae and all of 10 with folliculitis showed complete cure or marked improvement. Five of the patients with sycosis barbae and 1 with folliculitis required systemic oxytetracycline therapy because deeper skin structures were infected. Usually troublesome secondary infections associated with eczemas also responded dramatically. However, patients with acne vulgaris gave no evidence of improvement from the use of the ointment.

The author indicated that no local sensitivity reactions were observed in this series of cases. He concluded that the combination of oxytetracycline and polymyxin B in a petrolatum base is one of the best topical anti-infectives presently available.

The Effect of Antibiotics on the Incidence of Candida Albicans Infection in the Intestinal Tract of Mice. Cazin, M. H., Jr., and Smith, H., Jr. *J. Bact. 70:*440 (1955). With the advent of antibiotic therapy, it soon became evident that organisms resistant to the antibiotic frequently caused a secondary infection. *Candida albicans* is one of the more commonly encountered secondary invaders. In an attempt to learn the cause of superinfections with *C. albicans,* the authors studied the effect of orally administered antibiotics to mice when an oral suspension of *C. albicans* was also given orally. The following antibiotics were investigated: chlortetracycline,

chloromycetin, oxytetracycline, dihydrostreptomycin, magnamycin, erythromycin, penicillin, and tetracycline.

All of the antibiotics used favored the establishment of an intestinal population of the fungus. However, a previously postulated theory that the antibiotics directly stimulate the fungus cannot be the fundamental mechanism responsible for the overgrowth of *C. albicans*. This conclusion was drawn from the fact that stimulation of the fungus could be demonstrated *in vitro* or *in vivo* by only three of the antibiotics tested, namely, chlortetracycline, neomycin and bacitracin. Since it was found that all of these antibiotics favor the establishment of an intestinal flora of *C. albicans* in mice and since this seems to parallel the experience with antibiotics in humans, the authors suggested that this phenomena might be usable for evaluating the efficacy of prophylactic agents and possibly of chemotherapeutic agents in the control of secondary fungus infections concomitant with antibiotic therapy.

The authors also observed a marked difference in the susceptibility and consistency of response of the two strains of mice used to *C. albicans*. This points out the necessity of taking such differences into consideration when comparing the work of different investigators.

The Ultraviolet Radiation of Naturally Contaminated Water in the Process of Purification. Ricks, H. C., Cortelyou, J. R., Labecki, T. D., McWhinnie, M. A., Underwood, F. J., Semrad, J. E., and Reeves, G. R. *Am. J. Pub. Health 45:*1275 (1955). Ultraviolet radiation has been used as a means of reducing the bacterial content of water and thus rendering it potable or, perhaps, sterile. A small unit known as the A. R. F. U-V Water Purifier was used by the authors to study the basic factors which apply to the use of ultraviolet light in water purification.

They found that intensities of 650 mw/sq. ft. were effective at a distance of 2¾ in. in air for the destruction of the test organism for fecal contamination, *Escherichia coli*. A similar effect was obtained in water using the unit described. The lamp gradually decreases in the intensity of radiation emitted. With a lamp starting out with more than 100 mw/sq. ft., after 12 months of use for about 14 hours a day the emission was still above the 650 mw/sq. ft. indicated above. A reduction in line voltage and a decrease in temperature both decrease the intensity of radiation from the lamp.

However, a voltage even as low as 100 volts still provides 78 per cent of the original intensity while a temperature as low as 5° C. provides 87 per cent of the original intensity. Another factor affecting the available intensity is the mineral and organic content of the water.

Factors affecting the use of the available intensity of radiation were found to be variation in the resistance of organisms to the radiation, the duration of exposure, and the human element. The authors found that if *E. coli* was killed all common water-borne enteric pathogenic bacteria should also be destroyed. In the instrument used, the flow rate should not be more than 1½ qts. per min. to obtain adequate exposure.

A large series of field tests were also conducted. Following these studies the authors concluded that ultraviolet radiation constitutes an effective and safe means of purification of even heavily contaminated water provided a 2 minutes warm-up period is allowed, the water is reasonably clear by visual inspection, and the lamp is changed every 6 months. This is not intended to replace sound public health measures to provide safe water but should be useful in cases of emergency and in locations removed from supplies of known potable water.

The Use of Cortisone in Ulcerative Colitis. Truelove, S. C., and Witts, L. J. *Brit. Med. J.* No. 4947:1051 (1955). Cortisone was administered to a series of 109 patients with ulcerative colitis while 101 similar patients received a placebo. The dose of cortisone employed varied somewhat but the usual dose was 100 mg. a day for a period of six weeks. Two classes of patients were treated, those undergoing their first attack of ulcerative colitis and those undergoing relapses.

At the end of six weeks or the termination of therapy, whichever was shorter, 45 of the cortisone-treated patients had had remission of symptoms while only 16 of the control group had had remission. Those undergoing first attacks fared slightly better at this point than did those undergoing relapses, whether the condition of the disease was considered to be severe, moderate or mild. However, those who were severely ill at the beginning of treatment did not fare as well as those who were mildly ill. Complications were not significantly more severe among the cortisone-treated patients than among the control group, with the exception of pyogenic complications and eye

complications. The authors concluded that it is probably wise to administer sulfonamides or penicillin when cortisone is given in patients with ulcerative colitis.

In follow-up studies of these patients, it was found that at the end of nine months and at the end of the follow-up period, an average of about 18 months, the patients with first attacks who were treated with cortisone had maintained a definite edge over the corresponding control group. However, there was little difference between the two groups among those in relapse. It was also observed that at the end of the observation period there had been a general worsening of the condition of all patients. Therefore, the authors concluded that cortisone is a valuable adjunct in the treatment of ulcerative colitis, particularly among patients with first attacks. The effect is not, however, permanent, especially among those with an established disease.

A New Oral Dosage Form for Sulfonamides. Stephens, L. J., and Henrickson, W. E. *The Journal Lancet 75*:437 (1955). The fact that sulfonamides requires continuous administration at 4- to 6-hour intervals during periods of severe illness often leads to unfaithfulness in adhering to the prescribed schedule and to disturbance of the patient's rest. A new dosage form was studied by the authors which provided the sulfonamides in an oil emulsion vehicle designed to permit oral administration only twice a day.

The new dosage form had the following formula:

Sulfonamide(s)	2 or 10 per cent
Vegetable oil (highly emulsified)	50 per cent
Sucrose	12.5 per cent
Flavor and preservatives	q.s.
Water	q.s. ad 100 per cent

In experimental studies with mice the authors found that higher and more prolonged blood levels were obtained following the administration of the same dose of sulfadiazine in the emulsion vehicle as compared with administration in an aqueous vehicle. Four hours after administration the blood levels were about 28 and 16 mg. per cent and after 12 hours about 10 and 4 mg. per cent, respectively. It was also found that the oil emulsion caused the sulfadiazine to be

more effective in protecting mice from experimental hemolytic streptococcal infections. In human subjects these blood level findings were corroborated.

Triple sulfonamides in the emulsion vehicle were given to 53 patients with a variety of conditions. Uniformly, the response was favorable whether intended for prophylactic or therapeutic effects when the sulfonamides were administered in the oil emulsion at 12-hour intervals for 3 to 10 days, depending upon the condition. No toxic effects were noted. The authors, therefore, concluded that adequate sulfonamide medication can be provided by only two oral administrations a day when given in a fat emulsion vehicle.

BOOK REVIEW

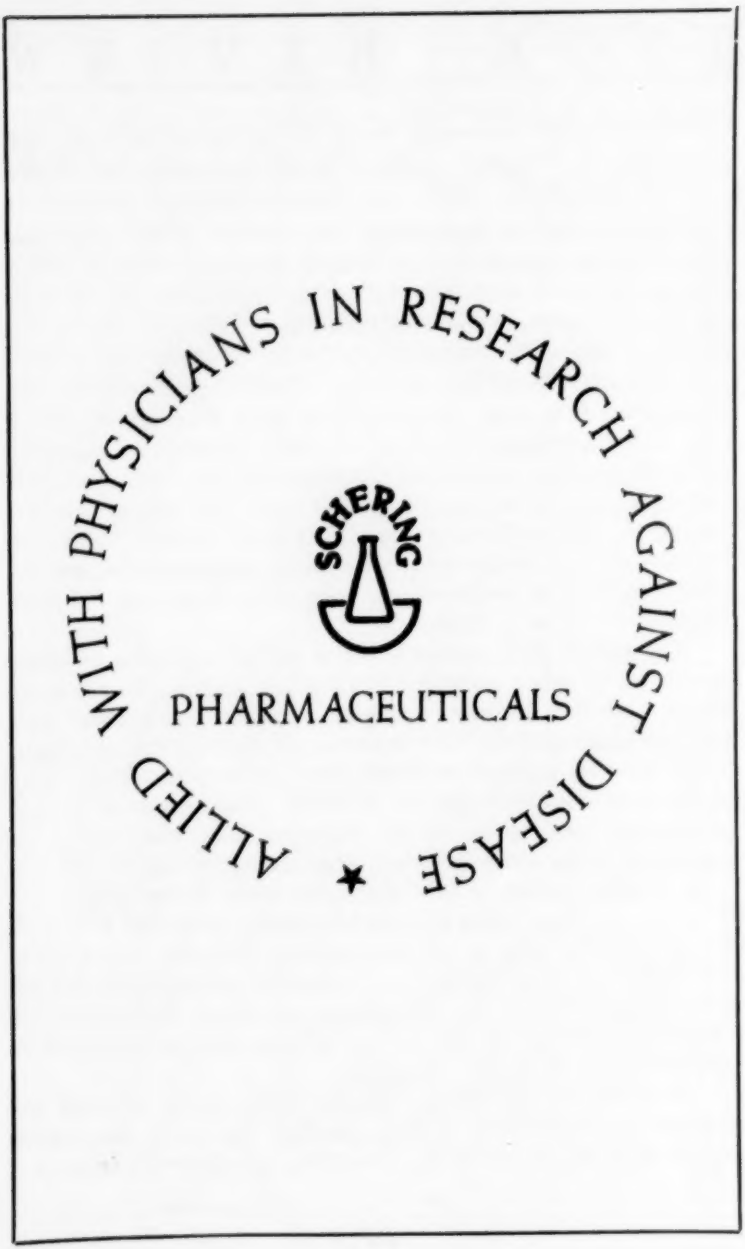
A French-English Dictionary for Chemists, Second Edition. By Austin M. Patterson. John Wiley & Sons, Inc., New York, 1954. 596 pages. $5.00.

This indispensable companion work to the author's same contribution in German was greatly in need of a newly revised edition as is its German counterpart. Filling the requirements of students and research scholars for a French-English chemical dictionary, this work lists an estimated 42,000 terms in chemistry, as well as in related sciences and industry. The inorganic and organic nomenclature is in conformance with the latest developments and is listed in the introduction in form of readily translatable examples. Technical words and phrases are labeled according to their specialized meaning in related sciences or general usage, the special chemical meaning of such words being placed first in the listing. Because of the many changes during recent years in the pharmacopeias, and because of the relative obsolescence of many older drugs, very old pharmaceutical terms are so indicated.

The author gives special attention to the numerous idiomatic phrases which, when translated literally into English, do not mean exactly what they indicate. Irregular verbs are listed under their own conjugational form with reference to their infinite parentage, so that when for example the French text lists the verb *doit*, the entry for the word indicates 3. sing. pr. of *devoir*. Many words of identical spelling and meaning in the two languages have been omitted for the sake of saving space; however, when ambiguity might occur, e.g. in the English endings of basic substances which in French may end in -in, or elsewhere, when the English ending, unlike the French, is -in, e.g. *palmitin, albumin,* etc., an entry has, of course, been made in the dictionary. Feminine forms of adjectives are generally omitted, but a complete key to their formation is included in the introduction. The same is the case for plural forms of adjectives and nouns which in French are frequently irregular.

A listing of chemical elements by their atomic numbers and symbols is unfortunately omitted, although the handy compactness of this dictionary is one of its outstanding and admirable features.

THEODORE L. LOWE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American Journal of Pharmacy

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